University of Wisconsin,
Madison

Dept. of Genetics,
March 24, 1949.

Dr. Luigi Cavalli,
Dept. Genetics,
University of Cambridge.

Dear Dr. Cavalli,

I am very pleased to hear that you have been able to get under way with your studies on recombination in E. coli K-12.

Your troubles with EMB agar are a little surprising. I am enclosing a packet with our dry mix of this formula, with which we have had no difficulty whatever. In our experience, K-12 has perhaps given a green sheen somewhat irregularly, but otherwise has always consistently given a strong # reaction on this medium, while Y-87 or W-583 which you have gives a very clearcut difference, remaining white or pink, barring reverse-mutations in the form of papillate outgrowths. Of course, these tests should be done at 37, and it occurs to me while writing that this may be the trouble. The formula we have used for EMB, which is also satisfactory for cross-streak phage resistance tests is: (per liter) Agar 15g., Lactose 10g.,,peptone (we use N-Z-Case) 8-10 g., yeast extract (not essential) 1g., dipotassium phosphate 2g., sodium chloride 5 g., methylene blue hydrochloride 0.065 g., Eosin Y 0.4 g.

I regret that my statement regarding the use of 30 should have caused confusion. 37 is perfectly satisfactory (and somewhat faster), although higher temperatures (ca. 40) are not, for all operations, and that is the only temperature I am now using. At Yale, owing to the Neurospora work, we had extensive constant temperature facilities for 30, which is why it was used.

Currently, I have been finding the following technic quite satisfactory. 10 ml. broth is inoculated from a reasonably fresh slant. The cells are harvested after growing overnight <u>without</u> shaking. Usually, they will be quite young enough, and a second growth cycle is not needed. The washed cells (saline is more satisfactory for washing than water, as the cells sediment more compactly) are mixed at a concentration of $10^9 - 10^{10}$ / ml., and .1 ml samples are spread with a bent glass rod on plates of synthetic agar (20 - 25 ml per plate). On the medium given in my paper, the prototrophs will appear within 24 - 36 hours at 37 C. We have rarely gotten yields of less than 10^{-7} under these conditions, although for reasons which are not understood, a particular run may give very poor results. But there should be no difficulty in reproducing the experiments. Of course in a cross of B$_1$ x TLB$_1$ the addition of thiamin (1 mg / liter is much more than enough) will increase the yields 10-fold by relaxing the conditions for segregation of B$_1$+. I certainly concur with you about the inadvisability of using ultra-violet light. I have not repeated the experiments, but have some question as to the possibility that the UV effect may be an artefact, perhaps due to recombination of lethals, or to the leakage of growth factors from the killed irradiated cells which will stimulate the growth of the survivors providing a larger population in which recombination might occur.

The trace elements are not needed, and may even be slightly inhibitory.

In my last letter, I mentioned the advantages of using a synthetic EMB medium (EMS). Our formula is: Agar 15, Sugar 10, Sod, Succinate 5, Ammon Sulfate 5, NaCl 1, $MgSO_4$ 1, and dipotass. phosph., and the dyes as above. Growth is rather slower on this medium than on the other synthetic ~~(Tris)~~ but prototrophs appear, and can be scored directly for their sugar character in 48 - 60 hours.

As stated in my Genetics 1947 paper, the mutation V_1^r carried by W-583 confers resistance to T1 and T5 (and T1h also). Other loci that have been identified include V_{1a}^r, resistant to T1, not to T1h or T5, and V_{1b}, a mucoid form with an ill-defined resistance spectrum, which is moreover unstable and quickly reverts to sensitive, non-mucoid. There is also V_1^P which appears to be an allele of V_1^r (no crossing over so far observed), which is partially resistant to T1, but almost completely sensitive to T5. However, the heterozygote V_1^r/V_1^P seems to be sensitive to T1, so this may be a very closely linked, non-allelic locus. These are all the T1-resistant phenotypes that I have found; there may be other loci involved which have not been ascertained.

As to the remainder of the resistance pattern: All T5-resistants seem to be V_{1a}^P or V_{1b}^r. V_2^r and V_6^r have been noted, but not studied except for the latter in my Genetics paper. There is also a " K/3,4,7" type which is slightly mucoid, but which gave me great trouble owing to instability either of the bacteria or of the phage, probably both. But there probably are some suitable T7-resistants. T3 (grown on B) is not active on most K-12 stocks, but contains a frequent mutant (ca 10^{-3}) which is. I have not found any "complex resistance" types such as Luria reports for B, but a " / 2, 3, 4, 6, 7 " type has been mentioned to me.

For genetic work, I would imagine that the V_1, V_{1a}, V_2, V_6, and V_7 mutations would be the most suitable. I am isolating a number of new coli phages for K-12, and if any distinct ones appear which give suitable resistant mutants, I will send them to you.

The W-583 stock which I sent you carries a Gal- mutation which is epistatic to Lac- (although satisfactory scoring is possible on 3% lactose). This probably will afford you no difficulty now, but I am sending W-677 which carries a different Gal-"slow" mutation which does not interact with Lac, as well as Mtl- (mannitol-negative). The arabinose-mutation is likewise a "slow" rather than negative. I have had no trouble in scoring any of these factors on EMB media. The constitution of W-677 is then:

T-L-B_1-V_1^r Lac_1- Mal_1- Gal- Ara- Xyl- Mtl- . I have very little information so far on their linkage relationships, beyond what is published. Gal and Lac are linked, with Ara not far distant; Xyl, Mal and Mtl are also closely linked, with Mal probably between B_1 and M, possibly between M and B. V_6, as published is slightly to the left of Lac_1, while V_1 is further to the right, nearer T and L.

For securing additional biochemical factors, I recommend using the "penicillin method", reprint enclosed. Other reprints are under separate cover. We now have quite a few other double biochemical mutants, e.g.,

W-758	isoleucine & valine; histidine	The isoleucine-valine, and
W-760	" " ; arginine	methionine-lysine requirements
W-828	histidine; proline (or glutamic)	are probably due to single gene
W-826	" ; serine (or glycine)	changes respectively.
W-832	methionine & lysine; tryptophane.	

For my own convenience, I will send some of these together with W-677. Do not hesitate to throw them away if you have no immediate use for them.

The diploid heterozygotes are still imperfectly understood. Outcrosses to these new stocks have not indicated that the aberrant segregations are due to some peculiarity of the older stocks, so the aberration may be uniquely connected with the persistence of the heterozygote (a ring-chromosome perhaps, or something of that order). Detailed crossover studies such as you contemplate would help a great deal to understand what is going on during segregation into prototrophs, and I will be very interested to hear of your findings. There still seems to be but a single chromosome.

With best regards,

Sincerely.

Joshua Lederberg